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DNA Interactions in Crowded Nanopores

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Abstract

The motion of DNA in crowded environments is a common theme in physics and biology. Examples include gel electrophoresis and the self interaction of DNA within cells and viral capsids. Here we study the interaction of multiple DNA molecules within a nanopore by tethering the DNA to a bead held in a laser optical trap to produce a 'molecular tug-of-war'. We measure this tether force as a function of the number of DNA molecules in the pore and show that the force per molecule decreases with the number of molecules. A simple scaling argument based on a mean field theory of the hydrodynamic interactions between multiple DNA strands explains our observations. At high salt concentrations, when the Debye length approaches the size of the counterions, the force per molecule becomes essentially independent of the number of molecules. We attribute this to the sharp decrease in electroosmotic flow which makes the hydrodynamic interactions ineffective.

KEYWORDS: nanopore, DNA, hydrodynamic interactions, crowding, optical tweezers

The electrophoretic tether force on single DNA molecules in solid state nanopores[1,2] is well studied by stalling DNA translocation using optical tweezers.[3–7] These investigations shed light on the complex interplay of hydrodynamic and electrokinetic effects governing DNA translocation.[8–13] However, in real systems such as biological cells or gel electrophoresis, DNA is often confined, and this crowding can alter the behavior of the molecules.[14,15] In the presence of non-electrical forces, the free-draining behavior of polyelectrolytes is replaced by one in which hydrodynamic interactions become dominant.[13,16–18] In this work, we study the interaction of DNA molecules with a combination of glass nanopores and optical tweezers.[7] A schematic of the setup is shown in Figure 1A (see Methods). A micron-sized polystyrene bead coated with double-stranded (ds) DNA molecules is held with an optical trap in close proximity to the glass nanopore using a piezoelectric nanopositioning system. The voltage applied across the nanopore drives a DNA molecule into the pore until the polymer is pulled taut and the bead displaced from its equilibrium position. The translocation process is eventually stalled indicating that the force applied by the optical trap (F_t) is in balance with the electrophoretic driving force (F_{EP}). A typical measurement is shown in Figure 2.

We are able to insert more than one DNA molecule into the same pore: this appears as consecutive and simultaneous step changes in force and current, with each step corresponding to an insertion event as shown in Figure 2. By monitoring the ionic current, it is possible to determine the total conductance change. We find this quantity to be proportional to the number of DNA molecules inside the pore, in agreement with previous free translocation studies, where the total conductance change indicated the folding state of the molecule.[19–22] Thus, the ionic current is a direct indicator of the number of DNA molecules inside the pore at any given time.

The stall force remains a linear function of voltage even with multiple insertions. This is shown in Figure 3A for $N = 1, 2$ and 3 DNA strands. It is therefore possible to define a voltage-independent measure of the stall force which is the gradient of the force-voltage graph. We call this quantity the scaled capture force, with units of pN/mV. However, unlike the conductance, the scaled capture force increases sub-linearly with the number of inserted DNA molecules N. This

is shown in Figure 3B, summarizing results from twenty experiments carried out on the same 100 nm-diameter pore in 500 mM KCl. We observe this behavior qualitatively for all pore sizes (diameter $= 100 - 580$ nm) and salt concentrations (20 mM$-$1 M KCl) that we considered. The degree of nonlinearity does however exhibit a dependence on salt concentration. We argue below that the observed dependence is due to hydrodynamic interaction between molecules.

Let us first consider the case of a single DNA molecule occupying the pore. For simplicity, a cylindrical geometry is assumed, although the results can be generalised to nanopores with slowly-varying cross section.[8] On account of the small diameter ($2R$) of the pore in comparison to its length (L), the problem may be considered as two dimensional. Thus, the DNA is regarded as a cylinder of radius a that is located within a larger cylinder (the nanopore) of radius R. In the experiments reported here $R/a \sim 50 - 100$. Although the DNA is electrostatically repelled from the cylinder wall, such interactions are shielded on a length scale of the order of the Debye length, $\lambda_D \sim a << R$. It is therefore reasonable to assume that as the DNA fluctuates within the pore due to Brownian motion, it explores the entire cross-section of the cylinder uniformly. Thus, the measured force is a statistical average of the forces that may be calculated by locating the DNA at various fixed points within the cylinder. If we assume that this average force (F_t) is the same as the force on a strand that is localized along the cylinder axis, then[9,10]

$$F_t = \frac{2\pi\mu L}{\ln(R/a)}(u_p - u_w) \tag{1}$$

where u_p and u_w are the Helmholtz-Smoluchowski slip velocities at the surface of the DNA and at the wall and μ is the fluid viscosity.

Let us now consider the situation where multiple DNA molecules are inserted in the pore. A DNA molecule held stationary within a nanopore will act as an electroosmotic pump due to the unbalanced charge in its counter ion cloud. Thus, if N DNA molecules occupy the nanopore, each one affects all the others by increasing the mean electroosmotic flux through the pore. The relevant forces in this situation are illustrated in Supplementary Figure S1.

One can calculate the average force per DNA molecule by using a mean-field approximation. The essence of this approximation is that eq 1 may still be used to calculate the average force on a DNA molecule, provided we replace u_w by $u_w + \Delta u_w$, where Δu_w is the perturbation of the uniform electroosmotic flow in the capillary due to the averaged effect of all neighbouring strands. In the limit of thin Debye layers, the mathematical problem of determining Δu_w clearly takes the following form: we must first determine the function u that satisfies

$$\nabla^2 u = 0 \tag{2}$$

within the circular cylinder of radius R with boundary condition $u = 0$ on the cylinder walls and $u = u_p - u_w$ at the locations of the DNA. Then $\Delta u_w = \overline{u}$ where the bar indicates a statistical average over all possible configurations of the DNA. Clearly, if $N = 1$ then $\Delta u_w = 0$ by definition. It is also clear that when N is large, Δu_w approaches a 'plug flow' profile of strength $(u_p - u_w)$. In the general case of a finite N, the perturbation Δu_w is approximately the flow perturbation experienced by a DNA molecule due to its nearest neighbor. This flow perturbation increases with N because the DNA molecules get more closely packed together and the distance to the nearest neighbor decreases. One could estimate the average distance between the DNA by imagining that the influence of each molecule extends to a circular patch of radius ρ and all such patches taken together span the whole cross-section. Thus, $N\pi\rho^2 \sim \pi R^2$ or $\rho \sim R/\sqrt{N}$. The flow perturbation can then be estimated by inserting $r = \rho$ in the formula for the flow perturbation at a distance r from a single DNA at the origin[8]

$$u(r) - u_w = (u_p - u_w)\frac{\ln(r/R)}{\ln(a/R)}. \tag{3}$$

Thus,

$$\Delta u_w = \overline{u} = -c(u_p - u_w)\frac{\ln N}{2\ln(a/R)} \tag{4}$$

where we have inserted a positive dimensionless constant c to acknowledge that this is only a rough scaling argument. On replacing u_w by $u_w + \Delta u_w$ in eq 1, where Δu_w is evaluated using eq 4, we can

predict the force per molecule when N molecules occupy the pore

$$F_t(N) = F_t(1)\left[1 - \frac{c\ln N}{2\ln(R/a)}\right]. \tag{5}$$

Here $F_t(1)$ denotes the force on a single molecule and is given by eq 1. In eq 4 the flow perturbation from a DNA molecule was evaluated in the limit of thin Debye layers. However, the final result for the force (eq 5) is valid independent of this approximation (see Methods).

To test the validity of the above theoretical arguments we performed a numerical simulation (see Methods) of the problem using the continuum representation of the fluid and ions in the pore. Figure 4C shows such a computed velocity distribution with three DNA molecules in the pore. The charged Debye layer surrounding each DNA molecule acts as an electroosmotic pump, and thus, the average electroosmotic flow increases with the number of molecules occupying the pore (see animation in Supplementary Figure S2). The normalized force per molecule, $F_t(N)/F_t(1)$, evaluated from the numerical solution is plotted against $\ln N/[2\ln(R/a)]$ in Figure 4D. The linear relation is satisfied very well for a range of different salt concentrations with $c \approx 1$, confirming the validity of the theoretical arguments leading to eq 5.

In Figure 4A, the experimentally measured values of $F_t(N)/F_t(1)$ are plotted as a function of $\ln N/[2\ln(R/a)]$ for different salt concentrations. As expected, the relationship is linear but the slope ($-c$) is seen to depend on salt concentration. This is not expected on the basis of eq 5 or from our numerical simulations. In order to understand the source of the discrepancy, the coefficient c at each salt concentration was extracted from a linear fit and plotted in Figure 4B as a function of the Debye length. We see that c appears roughly constant for $\lambda_D > 0.6$ nm but sharply drops to zero for smaller Debye lengths. That is, at high salt concentrations the non-linearity disappears, with a transition between the linear and non-linear regimes occurring at $\lambda_D \sim 0.6$ nm. The fact that the size of the hydrated K^+ ion is roughly 0.6 nm suggests a tentative explanation: for Debye lengths smaller than the size of the counterions, the continuum theory breaks down. Since the Poisson-Boltzmann theory assumes point-like particles, its failure to explain behaviour at length

scales smaller than the hydrated ion size is not surprising. In this limit, the counterions are confined to within a few ionic diameters of the DNA which seems to remove the electroosmotic forcing and hence the hydrodynamic interactions between the strands. Consequently, the nonlinear behavior disappears. The difference in the numerical values of c obtained from simulations and experiment could be due to the fact that our assumption that an individual DNA samples the available pore area uniformly may not be very accurate. This could be due to a combination of factors such as steric interactions, DNA rigidity, and the existence of correlations. In addition we assume that the DNA is a uniform cylinder neglecting any effects due to specific structural features of the double helix.

In conclusion, we have shown that multiple stalled DNA molecules in a nanopore couple to each other hydrodynamically. Experimentally, the effect of this is to reduce the tether force per molecule as more DNA molecules are added to the pore. This can be explained using a mean field theory, where neighboring molecules act as a perturbation to the background electroosmotic flow in the nanopore. The environment confining the translocating DNA is thus critical in determining the translocation force. This insight is important in understanding polymer translocation in confinement, such as in gels and in the crowded interiors of cells, and provides a complementary force-based approach compared to mean-field reptation theories. For high enough salt concentrations where the Debye length is comparable to the ionic radius, the continuum approximation breaks down and hydrodynamic flows decrease rapidly.

Materials and Methods

Experimental procedures

Our experimental setup consists of two primary components: the optical tweezers and the nanopore. The experimental design is similar to that in previous studies[3] except for the use of pores fabricated from glass nanocapillaries[19] and a laser orientation perpendicular to the capillary axis offering superior force resolution.[7] The optical tweezers are based on a custom-built inverted microscope

setup which uses a single-beam gradient optical trap, generated by focusing a ytterbium fibre laser beam at 1,064 nm wavelength (YLM-5-LP, IPG Laser, Germany) to a diffraction limited spot inside a microfluidic sample cell. This provides a stable three-dimensional trap which is used to capture ~ 2 μm-diameter polystyrene microparticles (Kisker, Germany), which are coated with dsDNA molecules (λ DNA, 48.6 kbp, New England Biolabs, USA) using streptavidin-biotin linkers. The position of the bead is tracked using a high-speed CMOS camera (MC1362, Mikrotron, Germany) at several thousand frames per second. Displacements of the bead's position from the centre of the trap are converted to forces by a power spectral density calibration method. The position of the trap is controlled using a piezoelectric nanopositioning system (P-517.3 and E-710.3, Physik Instrumente, Germany).

The sample cell consists of two reservoirs filled with salt solution and joined by a nanopore, and is constructed out of PDMS and sealed using a cover slide. Ag/AgCl electrodes are connected to each reservoir, and joined up to a commercial physiology amplifier (Axopatch 200B, Molecular Devices) for low-noise ionic current detection. Measurement solutions consist of varying concentrations of KCl buffered with 1x Tris-EDTA (TE) buffer (Sigma Aldrich) which maintains the solution close to pH 8. Both ionic current and position detection are performed simultaneously using custom-designed LabVIEW software.

Our nanopores are fabricated by pulling glass capillaries using a commercial pipette puller (P-2000, Sutter Instruments): this results in a conical-shaped glass nanopore with tuneable tip diameters between several tens to several hundreds of nanometers.[19] The size of the nanopore was characterized by measuring its current-voltage characteristics, or by direct imaging using a scanning electron microscope.

Numerical Simulation

The coupled Poisson-Boltzmann and Stokes equations were solved in an infinitely long pore (radius $R = 50$ nm) with a constant electric field applied in the axial direction. The DNA molecules were regarded as N uniformly charged cylinders placed parallel to the pore axis. The surface

charge on the pore was prescribed. The equations were solved using the COMSOL Multiphysics platform that uses a finite element solver. A fine mesh was embedded close to the DNA to increase the accuracy of the solution in the double layer. The tether force on each DNA molecule was found by subtracting the hydrodynamic drag force from the electric force on the charged cylinder. A new random distribution of the DNA centres was then chosen and the process repeated. Selections involving overlap of the molecules with each other or with the wall were rejected from the sample. The force was calculated by averaging the results over a large number of such separate computations.

Theory

Equation 5 was derived in the limit of thin Debye layers. However, we demonstrate here that it is valid irrespective of the Debye length or the size of the zeta potential as long as the pore may be considered an infinite cylinder. Computational modeling shows that when the pore geometry deviates from that of a uniform cylinder, the force does depend on the Debye length. However, this dependence is quite weak for typical pore shapes of relevance.

Consider the problem of N DNA molecules, modeled as charged cylinders, positioned at locations $\mathbf{r}_i$ within a uniform capillary of radius R and large length. The electroosmotic flow in the axial direction (z) is $\mathbf{u} = \hat{\mathbf{z}}u(x,y)$ where $\hat{\mathbf{z}}$ is the unit vector along z. The velocity obeys Stokes equation with an electric body force

$$\mu\nabla^2 u + \rho_e E_0 = 0 \tag{6}$$

where $\mathbf{E} = \hat{\mathbf{z}}E_0$ is the applied electric field, ρ_e is the free charge density and $\nabla^2 = \partial_x^2 + \partial_y^2$ is the two dimensional Laplacian. The free charge density is related to the equilibrium potential ϕ by Poisson's equation:

$$\varepsilon\nabla^2\phi = -\rho_e, \tag{7}$$

ε being the permittivity of the medium. If we define the new variable

$$w = u - \frac{\varepsilon E_0}{\mu}\phi \tag{8}$$

then w satisfies

$$\nabla^2 w = 0. \tag{9}$$

If the classical 'no slip' flow boundary conditions are assumed

$$w = -\frac{\varepsilon E_0}{\mu}\phi_w = u_w \quad \text{at walls} \tag{10}$$

$$w = -\frac{\varepsilon E_0}{\mu}\phi_p = u_p \quad \text{at DNA surface,} \tag{11}$$

where ϕ_p and ϕ_w are the values of ϕ on the DNA and on the wall and u_p and u_w are the corresponding Helmholtz-Smoluchowski slip velocities. Formally, w is the velocity field one would get if the Debye layers were regarded as infinitely thin and the classical Helmholtz-Smoluchowski slip boundary conditions were applied. If w is somehow known, the velocity u can be determined by inverting eq 8

$$u = w + \frac{\varepsilon E_0}{\mu}\phi. \tag{12}$$

The viscous force on the DNA molecules is then given by

$$\begin{aligned} F_v &= \mu L \oint \nabla u \cdot \hat{\mathbf{r}}\, ds \\ &= \mu L \oint \nabla w \cdot \hat{\mathbf{r}}\, ds + \varepsilon E_0 L \oint \nabla\phi \cdot \hat{\mathbf{r}}\, ds \end{aligned} \tag{13}$$

where $\hat{\mathbf{r}}$ is the radial unit vector centred on the DNA axis, L is the pore length and $\oint$ is evaluated around the contours of all the DNA molecules. The electric force on the DNA is

$$F_e = -\varepsilon E_0 L \oint \nabla\phi \cdot \hat{\mathbf{r}}\, ds. \tag{14}$$

Therefore, the tether force is given by

$$F_t = -F_e - F_v = -\mu L \oint \nabla w \cdot \hat{\mathbf{r}}\, ds. \tag{15}$$

This is exactly the force one would calculate in the limit of an infinitely thin Debye layer, because in this limit the DNA is electrically neutral and the flow field is described by $w(x,y)$.

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Supporting Information Available

A diagram showing the force balance during stalled translocation; an animation of the computed hydrodynamic solution. This material is available free of charge via the Internet at `http://pubs.acs.org/`.

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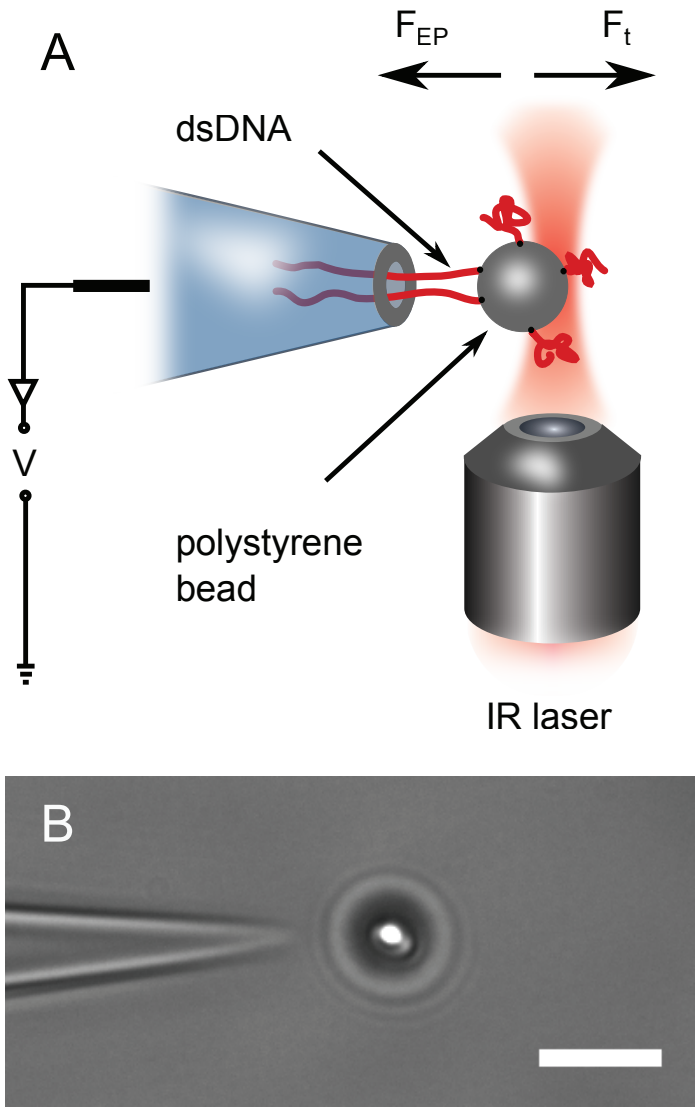

Figure 1: Combined optical tweezers and nanopore setup. (A) A schematic of the experimental geometry: the bead is in equilibrium under the influence of the electrophoretic force F_{EP} and the optical tweezers tether force F_t. (B) A micrograph showing an experiment in progress with a trapped DNA-coated bead in front of a glass nanopore. The scale bar represents 5 μm.

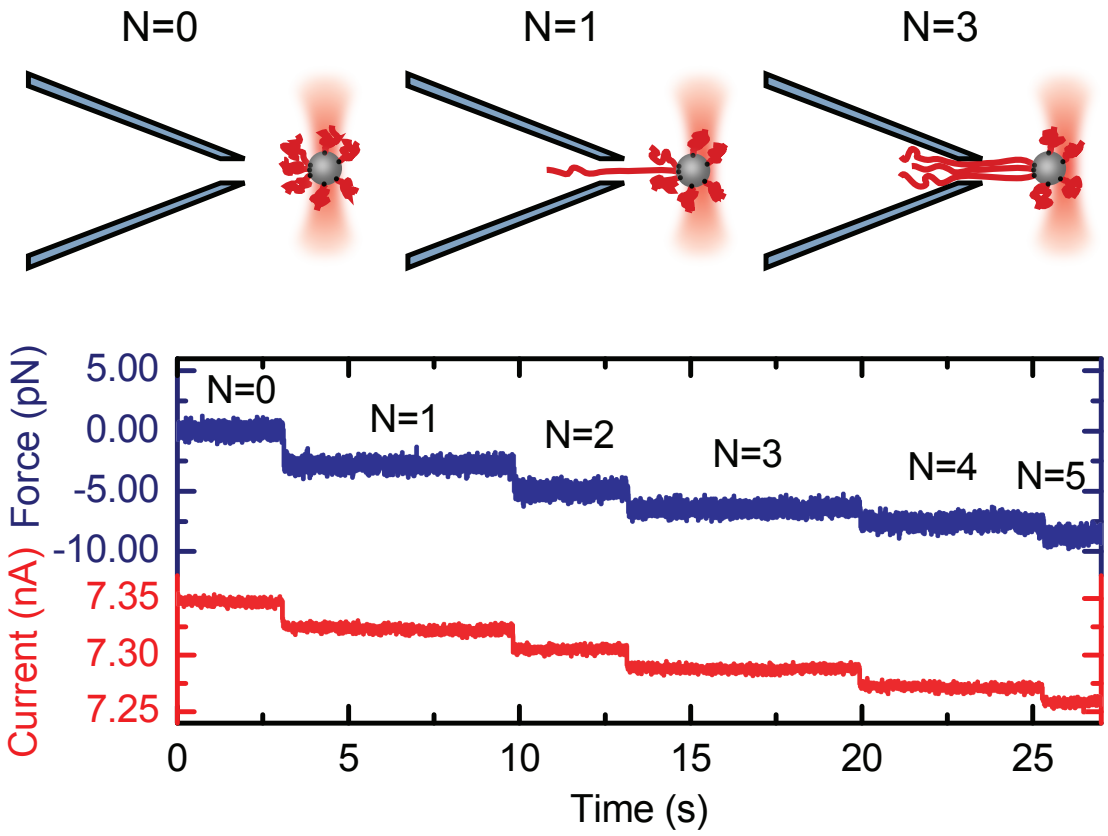

Figure 2: Insertions of multiple DNA molecules monitored by force and current. Time traces of force (blue) and ionic current (red) during successive insertions of up to five DNA molecules into a 100 nm pore, in 500 mM KCl. Each insertion event is characterised by simultaneous force and current steps. Experimental conditions are 500 mM KCl, 10 mM TE buffer pH 7.8.

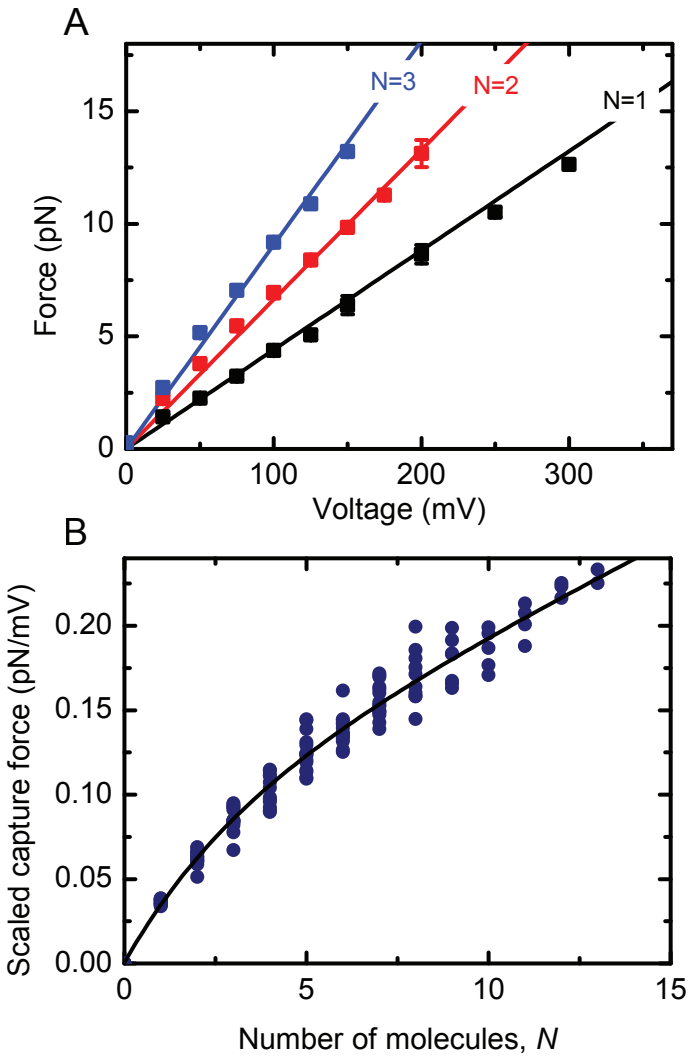

Figure 3: Scaled capture force increases non-linearly with the number of molecules. (A) The stall force is a linear function of voltage, even when multiple DNA molecules are inserted. Here, the force-voltage relationship is shown for up to three molecules in a 100 nm pore, in 100 mM KCl, 10 mM TE buffer pH 8. Error bars represent the standard deviation of a number of measurements. (B) The scaled capture force has a sub-linear dependence on the number N of DNA strands occupying the pore. The results of twenty experiments are shown, which were carried out in a 100 nm pore in 500 mM KCl, 10 mM TE buffer pH 7.8. The solid line is a fit to guide the eye.

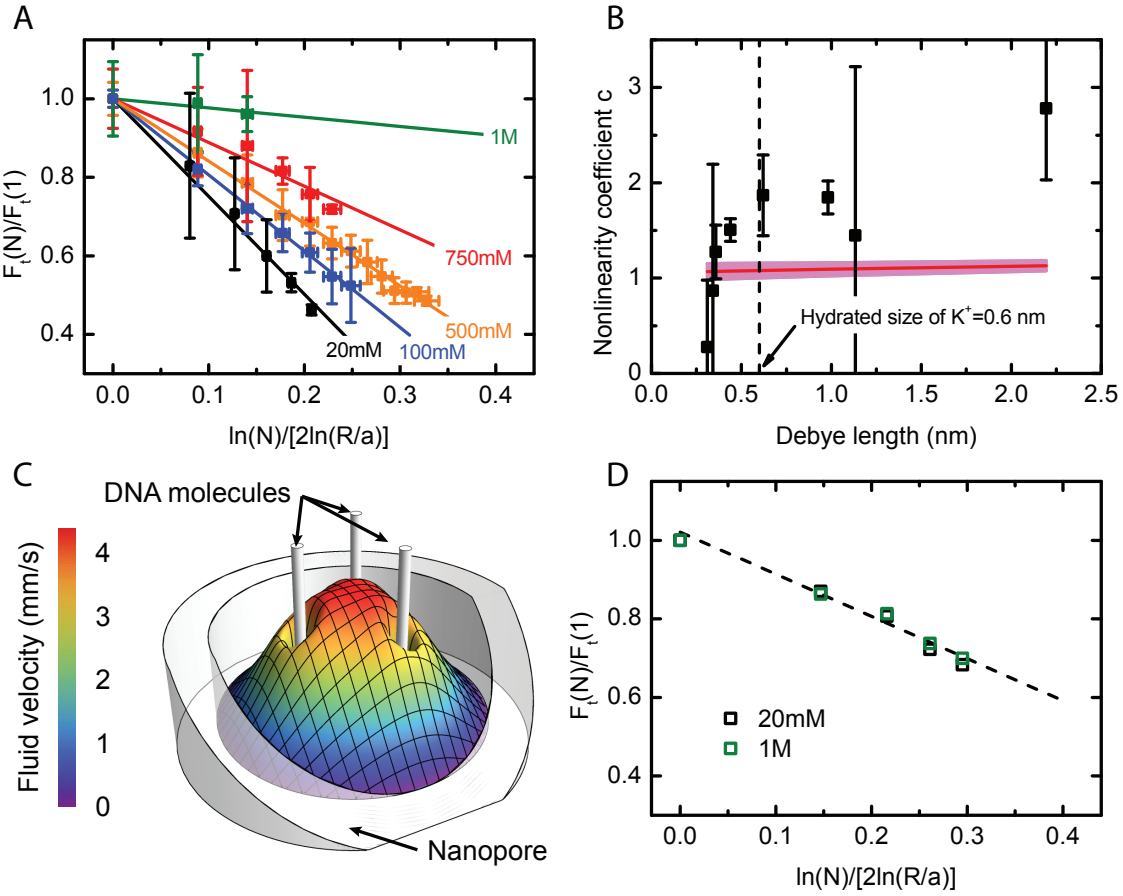

Figure 4: Hydrodynamic coupling enhances fluid drag. (A) The normalised force per strand $F_t(N)/F_t(1)$ plotted against $\ln N/(2\ln(R/a))$ gives straight lines with slope $-c$ in accordance with the scaling law given in eq 5. Error bars indicate the standard deviation of measurements (y-direction) and the uncertainty in pore size (x-direction). Data sets at 50 mM, 250 mM, and 825 mM are excluded for clarity. (B) The coefficient c plotted as a function of the Debye length. The error bars were determined from uncertainty in the slope of the fitted line. The horizontal line represents the value of c determined using the the computed solution with the width of the band representing uncertainty in its value. (C) The computed axial hydrodynamic velocity profile inside a pore with three inserted DNA molecules. The electroosmotic pumping by each molecule enhances the flow strength around its neighbors thereby increasing the frictional drag. (D) The normalized force per strand $F_t(N)/F_t(1)$ plotted against $\ln N/[2\ln(R/a)]$ as obtained from numerical simulation. The error is represented by the size of the points. Calculations were done at five different salt concentrations; however only two are shown for clarity. Unlike in the case of the experiment, c is found to be independent of salt concentration.

Graphical TOC Entry